

## Chemical Markers in the Aroma Profiles of South Moravian Red Wine Distillates

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### Abstract

VEVERKA L., JELÍNKOVÁ M., HRON K., BALÍK J., STÁVEK J., BARTÁK P. (2012): **Chemical markers in the aroma profiles of South Moravian red wine distillates**. Czech J. Food Sci., **30**: 369–376.

HSSPME-GC/MS method was used to investigate the volatile compounds responsible for varietal character in the aroma of wine distillates made from 16 different red wine grape cultivars: Andre, Blue Frankish, Merlot, Cabernet Moravia, Rubinet, Pinot Noir, Ariana, Alibernet, Laurot, Dornfelder, Blauer Portugieser, Agni, Neronet, Zweigeltrebe, Cabernet Sauvignon, and Domina. The grapes were all grown in the same vineyard in South Moravia, an important viticultural region in the south of the Czech Republic bordering Austria. The isometric log-ratio transformation was used to compute variances prior to statistical analysis, and a compositional biplot was used to interpret the data and identify the main chemical markers. A comparison of the key terpenoids present in the aroma profiles indicated that these were consistent with the known relationships between the cultivars based on their parentage. There were similarities in the terpenoid elements of the aroma profiles of Blue Frankish and its relatives Andre, Laurot, Agni, and Zweigeltrebe, which are dominated by (*Z*)-linalool oxide, linalool, isoborneol, terpinen-4-ol and  $\alpha$ -terpineol. On the other hand, the aroma profiles of Pinot Noir, Blauer Portugieser, Cabernet Sauvignon and their related hybrids are dominated by *o*-cymene, limonene, (*E*)-sabinyl acetate, and (*E*)-calamenene.

**Keywords:** terpenoids; compositional data; principal component analysis; centred log-ratio transformation; gas chromatography/mass spectrometry; solid-phase microextraction

Wine spirit, according to Regulation (EC) No. 110/2008 of the European Parliament and of the Council of the European Union, is a spirit drink produced exclusively by distillation at less than 86% vol. of wine or wine fortified for distillation or by the redistillation of a wine distillate at less than 86% vol. The minimum alcoholic strength by volume of

wine spirit shall be 37.5% (Ministerstvo zemědělství 2008). Wine spirits contain, apart from alcohol, a quantity of volatile substances which form the basis of their aroma. This aroma is composed of many different compounds which can, however, be assigned to one of the three groups according to their source of origin (RAPP 1998; TEŠEVIĆ *et*

Supported by Ministry of Education, Youth and Sports of the Czech Republic, Projects No. MSM 6198959214 and No. MSM 6198959216, and Palacký University, Czech Republic, Project No. PRF.2011.025.

*al.* 2005). Primary aromas, also known as grape aromas, are made up of aromatic compounds present in the undamaged plant cells of the grape. These compounds, represented mainly by terpenoids, are released into the wine during fermentation (KOSLITZ *et al.* 2008) and are captured during the subsequent distillation process. The precise chemical composition depends on the particular variety of grapevine and also on the degree of maturity (FLAMINI 2005; SÁNCHEZ PALOMO *et al.* 2007). Secondary aromas come from a group of higher alcohols, esters, volatile acids, aldehydes, ketones, etc. (LUKIĆ *et al.* 2006) and form the majority part of the spirit aroma. These compounds are generated by chemical, enzymatic and thermal reactions during technological processing of grapes and subsequent fermentation process. Tertiary aromas are generated during the process of distillation and subsequent maturation of the distillate. They are represented, for example, by a group of acetals (WILLIAMS *et al.* 1975) and compounds arising by the chemical reaction under heating conditions (e.g. heterocyclic compounds such as furfural, MADRERA *et al.* 2003).

For the identification and measurement of the compounds responsible for the aroma of wine distillates and other spirits, it is obviously necessary to use an appropriate extraction method, due to the complexity of the sample and also the low concentrations of several volatile compounds. Currently, conventional techniques such as liquid-liquid extraction (RAPP & GUNTERY 1985; RAPP *et al.* 1993; TEŠEVIĆ *et al.* 2005) or solid-phase extraction (LÓPEZ *et al.* 2002; LUKIĆ *et al.* 2006; CASTRO *et al.* 2008) are often replaced by solid-phase microextraction (BONINO *et al.* 2003; SÁNCHEZ-PALOMO *et al.* 2005; PROSEN *et al.* 2007; KOSLITZ *et al.* 2008). This rapid, simple and inexpensive technique for extraction and pre-concentration completely eliminates the need to use hazardous organic solvents in the extraction process.

In this paper, 16 distillates of wine made from red grape cultivars, grown in the same vineyard in South Moravia (Czech Republic), were analysed using solid-phase microextraction (SPME) coupled to gas chromatography/mass spectrometry to identify the important volatile compounds which together make up their aroma profiles.

## MATERIAL AND METHODS

**Samples.** Samples of 16 different varietal wine distillates produced from red wine grape cultivars

were used for this investigation. All the cultivars, namely Andre, Blue Frankish, Merlot, Cabernet Moravia, Rubinet, Pinot Noir, Ariana, Alibernet, Laurot, Dornfelder, Blauer Portugieser, Agni, Neronet, Zweigeltrebe, Cabernet Sauvignon, and Domina, originated from the experimental station in Oblekovice, which belongs to the Central Institute for Supervising and Testing in Agriculture in Brno, Czech Republic. Grapes were mashed and then fermented in plastic fermentative containers at a temperature of 15–17°C for 11 days. Crushed grapes were pressed afterwards and well-fermented wine was kept in glass containers. Neither yeast nor pectolytic enzymes were used. These young wines were used for production of wine distillates. The distillation process itself took place in a laboratory glass distilling apparatus. All of these wine distillates were produced using the same method from particular reference varietal wine by repeated (two-step) distillation. Final product was diluted to 47% ethanol.

**Instrumentation.** The volatiles of the wine distillates were extracted using HS-SPME and analysed by gas chromatography/mass spectrometry (GC/MS). HS-SPME results were obtained when the divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50/30 µm) coated fibre was inserted into a 35-ml septum-sealed flask containing 3 ml of wine distillate and 7 ml of saturated NaCl solution (p.a. purity; Lachema, Brno, Czech Republic), and exposed in the headspace for 60 min at controlled laboratory temperature (23°C). Analytes were desorbed in the injector of the gas chromatograph for 10 min (preventing any possible memory effect). Analyses were performed on an HP 7890A Series GC system with an Agilent 5975C Mass Selective Detector (Agilent, Palo Alto, USA), equipped with an HP-5MS capillary column (30 m × 0.25 mm I.D. × 0.25 µm film thickness). The details of the experimental conditions were as follows: desorption temperature 250°C, 10 min; splitless injection, temperature program 50°C – 3°C/min – 250°C (5 min), carrier gas was helium (99.998%; flow rate 0.9 ml/min; SIAD, Bergamo, Italy). MS scans were recorded in the range of 29–370 *m/z* using electron ionization (energy 70 eV). The volatile compounds were identified on the basis of their mass spectra by using the NIST 08 mass spectra library and by comparison of their calculated retention indices with the indices from literature (ADAMS 2004). Retention indices of volatiles were determined on the basis of the retention times of *n*-alkanes

C5–C20 (Sigma-Aldrich, St. Louis, USA). The repeatability of the experimental method was determined by performing three replicate analyses of each sample of varietal distillate. Calculated relative standard deviations (RSD%) of peak areas were less than 5%. Levels of volatile compounds in varietal wine distillates were expressed as the average values in relative units ( $\times 10^5$ ).

**Statistical analysis.** The data were processed using R programming language and software environment (R Development Core Team 2011a), which is nowadays one of the most commonly used and most popular software tools for statistics and data analysis. It is a free and open source (GPL 2) and it can be downloaded for all computer platforms from the comprehensive R archive network (R Development Core Team 2011b). It is enhanceable via packages which consist of code and structured standard documentation explaining the input and output arguments of each function including code application examples. Recently, two packages for compositional data have been available: “compositions” and “robCompositions”;

the latter (TEMPL *et al.* 2011) was used to perform the statistical analysis in the present paper.

## RESULTS AND DISCUSSION

In this study, wine distillates made from 16 different varietal red wines were analysed in order to identify the variety-specific aroma compounds. The volatile compounds identified by HSSPME in the wine distillates are shown in Figure 1 and Table 1. Since all the grapes came from the same region and the winemaking techniques were identical in each case, variations in the formation of secondary aromas were not considered since their contents depend on the production parameters more significantly. Therefore it is the primary aroma components, represented predominantly by terpenoids, which can be used to characterise individual grape varieties. Terpenoids have been reported as being the important varietal aroma compounds for the sensory perception of grapes (SCHREIER *et al.* 1976; WILLIAMS *et al.* 1980; ZALA-

Table 1. Content of terpenoids present in varietal wine distillates by HSSPME–GC/MS

Wine distillate	Aromatic compounds <sup>a</sup>									
	<i>o</i> -cymene	limonene	( <i>Z</i> )- $\beta$ -ocimene	( <i>Z</i> )-linalool oxide	linalool	isoborneol	terpinen-4-ol	$\alpha$ -terpineol	( <i>E</i> )-sabinyl acetate	( <i>E</i> )-clamenene
1. Andre	1.87	4.84	3.88	7.34	207.64	6.88	8.54	50.40	7.75	2.25
2. Blue Frankish	1.39	2.87	2.84	4.33	117.91	3.63	8.93	17.86	5.59	2.43
3. Merlot	0.77	2.89	1.90	3.68	90.56	4.80	5.22	11.25	1.24	2.84
4. Cabernet Moravia	8.81	19.07	2.75	2.59	54.98	2.98	2.29	16.00	3.74	4.18
5. Rubinet	6.20	11.72	4.19	5.89	72.54	3.38	8.83	27.38	2.93	2.08
6. Pinot Noir	3.52	7.76	2.44	1.91	41.62	2.60	2.21	10.84	3.88	3.47
7. Ariana	2.79	3.98	3.49	8.33	56.40	1.45	8.61	51.31	7.73	12.30
8. Alibernet	3.72	5.02	2.26	5.95	49.94	2.00	16.78	30.74	5.49	2.88
9. Laurot	1.48	0.89	1.15	18.63	45.41	1.19	20.88	70.56	5.33	35.14
10. Dornfelder	2.41	4.45	2.35	2.54	71.87	2.73	4.44	28.89	4.85	3.10
11. Blauer Portugieser	1.80	5.33	1.98	3.19	35.34	1.79	3.43	8.46	6.36	2.55
12. Agni	1.86	1.11	1.73	8.17	85.32	2.87	6.18	48.05	7.22	4.81
13. Neronet	1.64	3.22	<0.20	4.72	33.66	< 0.20	7.14	27.74	8.68	3.71
14. Zweigeltrebe	1.38	0.64	<0.20	13.29	30.93	1.50	9.89	52.77	7.95	4.33
15. Cabernet Sauvignon	1.57	1.10	<0.20	5.66	15.36	< 0.20	9.68	17.44	7.32	3.90
16. Domina	1.15	1.89	1.28	4.27	9.46	< 0.20	6.63	17.42	7.03	7.73

<sup>a</sup>mean values of volatile compounds expressed in relative units of the peak area ( $\times 10^5$ ) calculated for three replicates (RSD  $\leq 5\%$ )

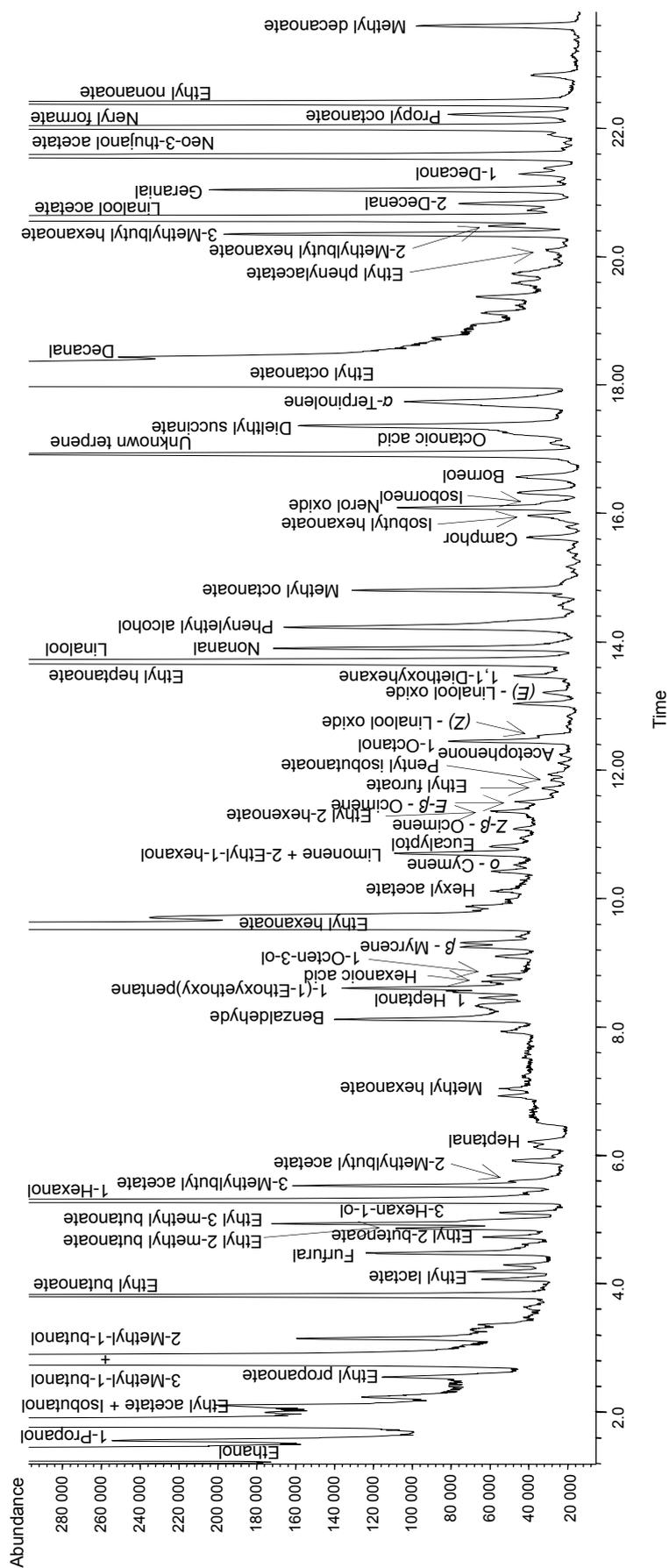


Figure 1. HSSPME-GC/MS chromatogram of Andre wine distillate

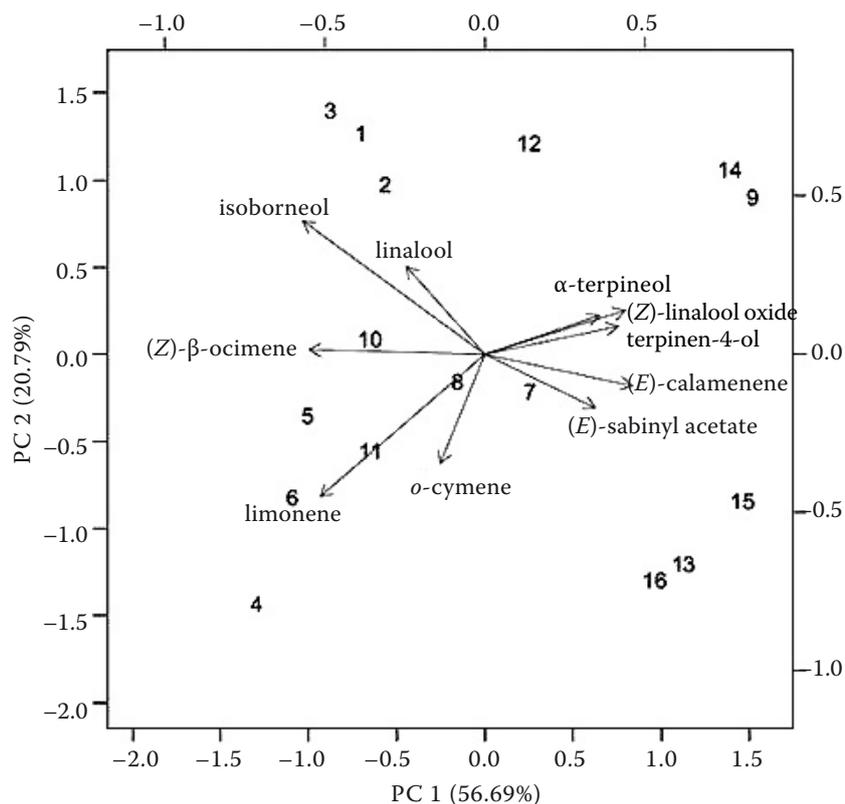


Figure 2. Compositional biplot for the wine distillate data (PCA for clr transformed data): numbers represent particular varietal wine distillates

1 Andre; 2 Blue Frankish; 3 Merlot; 4 Cabernet Moravia; 5 Rubinet; 6 Pinot Noir; 7 Ariana; 8 Alibernet; 9 Laurot; 10 Dornfelder; 11 Blue Blauer Portugieser; 12 Agni; 13 Neronet; 14 Zweigeltrebe; 15 Cabernet Sauvignon; 16 Domina

CIAN *et al.* 2007; SKINKIS *et al.* 2008), wines (RAPP *et al.* 1985; RAPP 1998) and their related distillates (LILLO *et al.* 2005) including grape pomace distillates (DIÉGUEZ *et al.* 2003; LUKIĆ *et al.* 2006, 2010; LÓPEZ-VÁZQUEZ *et al.* 2010).

In situations such as ours, where the data carry only relative information (compositional data, as opposed to standard multivariate observations), the usual statistical methods cannot be applied directly to the raw data, because it often leads to

Table 2. Overview of the varieties and their origins ([www.wineofczechrepublic.cz](http://www.wineofczechrepublic.cz))

Cultivars	Parent 1	Parent 2
1. Andre	Blue Frankish	St. Laurent
2. Blue Frankish		
3. Merlot	genetically related to Cabernet Franc variety	
4. Cabernet Moravia	Cabernet Franc	Zweigeltrebe
5. Rubinet	Revolta × Alibernet	Andre
6. Pinot Noir		
7. Ariana	Riesling × St. Laurent	Zweigeltrebe
8. Alibernet	Alicante Bouschet	Cabernet Sauvignon
9. Laurot	Merlot × Seibel 1366	Andre
10. Dornfelder	Helfensteiner × Heroldrebe	Blauer Portugieser × Blue Frankish
11. Blauer Portugieser		
12. Agni	Andre	Irsai Oliver
13. Neronet	St. Laurent × Blauer Portugieser	Alibernet
14. Zweigeltrebe	St. Laurent	Blue Frankish
15. Cabernet Sauvignon	Cabernet Franc	Sauvignon
16. Domina	Blauer Portugieser	Pinot Noir

meaningless results. Therefore so-called log-ratio transformations were used to make a standard statistical analysis possible (AITCHISON 1986). To compute variances and identify main markers among the compounds, the isometric log-ratio transformation (EGOZCUE *et al.* 2003) was applied first (resulting in variables that contain all the relative information concerning the compounds of interest) and the selected compounds were analysed using a compositional biplot (KORHOŇOVÁ *et al.* 2009). The method of greatest variance was applied to the corresponding isometric log-ratio variables to identify dissimilarities in the aroma constituents of the red grape cultivars. The compositional biplot (Figure 2) shows ten terpenoids which are suggested as potentially useful markers: *o*-cymene, limonene, (*Z*)- $\beta$ -ocimene, (*Z*)-linalool oxide, linalool, isoborneol, terpinen-4-ol,  $\alpha$ -terpineol, (*E*)-sabinyl acetate, and (*E*)-calamenene.

Further statistical evaluation separated the particular wine distillates into the different quadrants of the compositional biplot. 77.48% of the total variance of the initial data, defined by 10 variables (terpenoids) and 16 objects (wine distillates), could be explained by a combination of the first principal component (PC1), which accounts for 56.69%, and the second principal component (PC2), which accounts for 20.79%. Distribution of distillates into particular groups is based on their association with specific aroma compounds and the proportions of terpenoids in each cultivar. Most of the grapevine varieties have been created by the hybridisation of just a few older wine cultivars (Table 2), so it is to be expected that grapevine varieties with a common background will contain similar compounds in similar proportions, and that this pattern will also be seen in the corresponding wine distillates. Blue Frankish, St. Laurent and their relatives, which are strongly linked by linalool, isoborneol, (*Z*)-linalool oxide,  $\alpha$ -terpineol and terpinen-4-ol, are located in the top part of the biplot. However, Laurot and Zweigeltrebe are associated with just (*Z*)-linalool oxide,  $\alpha$ -terpineol, and terpinen-4-ol, and Andre and Blue Frankish with just linalool and isoborneol. The terpenes linalool and isoborneol also strongly influenced the aroma profile of the Merlot wine distillate. On the other hand, wine distillates based on Cabernet Sauvignon, Pinot Noir, and Blauer Portugieser were strongly correlated with the presence of *o*-cymene, limonene, (*E*)-sabinyl acetate, and (*E*)-calamenene, and are placed in the lower part of the plot. The second

Table 3. Compounds studied and their aromatic characteristics (Leffingwell, Flavornet)

Compound	Descriptors
Calamenene	herbs, spices
<i>o</i> -Cymene	solvent, gasoline, citrus
Isoborneol	camphoraceous, sweet, musty
Limonene	fresh citrus
Linalool	lavender, sweet, rose
Linalool oxide	sweet, woody, floral
Ocimene	herbaceous, fruity, floral
Sabinyl acetate	balsamic
Terpinen-4-ol	terpentine, musty, nutmeg
$\alpha$ -Terpineol	peach, aniseed, fruity, lily of the valley

principal component (PC2) could be regarded as a discriminator for properties of the variety. However, the volatile compounds in some grapevine varieties are different, or are present in different proportions, when compared to their relatives (SKINKIS *et al.* 2008), and this was observed to be the case for the distillate prepared from the cultivar Domina, a cross between Blauer Portugieser and Pinot Noir, which was placed on its own.

The observed profiles of aroma compounds in the distillates made from these wine varieties are in line with other studies, especially on grapes and wines (RAPP & GUNTER 1985; RAPP *et al.* 1993; RAPP 1998; FERRARI *et al.* 2004; PROSEN *et al.* 2007; SKINKIS *et al.* 2008; MASON *et al.* 2009; LUKIĆ *et al.* 2010). The relative amounts of primary aroma compounds, which are primarily responsible for differentiating pure varietal wine distillates, are also responsible for determining either the spicy or floral character of each compound and also the intensity of its aroma (Table 3). Spiciness, defined as possessing the aromas of aniseed, cloves, nutmeg or ginger, is an indication of the presence of terpinen-4-ol,  $\alpha$ -terpineol, (*E*)-sabinyl acetate and (*E*)-calamenene, respectively. Floral and sweet aromas, on the other hand, are the result of the presence of linalool, (*Z*)- $\beta$ -ocimene or limonene.

## CONCLUSIONS

Centred log-ratio transformation and principal component analysis were applied in the analysis of varietal wine distillates. Ten terpenoids were identified as the principal contributors to the

characteristic aroma of the grapevine cultivars: *o*-cymene, limonene, (*Z*)- $\beta$ -ocimene, (*Z*)-linalool oxide, linalool, isoborneol, terpinen-4-ol,  $\alpha$ -terpineol, (*E*)-sabinyl acetate, and (*E*)-calamene. Statistical evaluation of the data by principal component analysis demonstrated similarities between the wine distillates, when the aromas of cultivars Merlot, Blue Frankish and their respective hybrids Andre, Laurot, Agni, and Zweigeltrebe were strongly influenced by (*Z*)-linalool oxide, linalool, isoborneol, terpinen-4-ol,  $\alpha$ -terpineol, and the aromas of Pinot Noir, Blue Blauer Portugieser, Cabernet Sauvignon and their hybrid cultivars were strongly influenced by the terpenoids *o*-cymene, limonene, (*E*)-sabinyl acetate, and (*E*)-calamene.

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Received for publication May 16, 2011

Accepted after corrections October 19, 2011

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